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NCIC HPV
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Subject: Comments on the ACC's test plan for DAEs

03/25/2003 02:55 PM

Jessica Sandler <jessicas@peta.org> on 03/25/2003 12:59:36 PM

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cc:

Subject: Comments on the ACC's test plan for DAEs

Attached please find the comments of the American animal protection community on the ACC's HPV test plan for DAEs.

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HPV test plan comments -- DAE.pdf

201-14370

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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March 25, 2003

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building (1101A)
1200 Pennsylvania Ave., NW
Washington, DC 20460

Re: Comments on the HPV test plan for dithiophosphate alkyl esters

Dear Administrator Whitman:

The following are comments on the test plan for the category dithiophosphate alkyl esters (DAEs), prepared by the Health, Environinental and Regulatory Task Group (HERTG) of the American Chemistry Council's Petroleum Additives Panel. These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Coniniittee for Responsible Medicine (PCRM), the Humane Society of the United States, the Doris Day Animal League, and Earth lsland Institute. These health, animal, and environmental protection organizations have a combined membership of more than ten million Americans.

Firstly, we would like to commend the test plan in several respects:

(a) *It recognizes the importance of limiting animal testing*

The test plan contains the following statements:

> animals will experience extreme pain and suffering with additional testing. (p. ii)
>
> additional repeated-dose and reproduction and developmental testing would only cause distress and suffering in experimental animals and would add no additional insight into the risk of adverse health effects. (p. iii)
>
> careful consideration was given to the number of animals that would be required for tests included in the proposed plan and conditions to which animals might be exposed. In consideration of some non-governmental organizations about animal welfare, the use of animals in this proposed test plan has been minimized. (p. iv).

(b) *It uses chemical categories*

HERTG has included nine coinpounds (CAS nos. 84605-28-7, 685 1-01-8, 68784-30-5, 1 13706-14-2, 6028-47-3, 5810-88-8, 68649-43-4 and 26999-29-1) in a single category, termed DAEs. Data are thus only required for the category as a whole. The test plan presents a detailed justification for categorization (pp. i-ii), on the basis of the following types of similarity between the nine compounds: (i) molecular structure (each consists of phosphorodithioic acid with two alkyl ester substituents; **pp.** 3-4, 20); (ii) physicocheinical properties (all coinpounds have molecular weights of 256-354 daltons, are highly acidic

and lipophilic, and have low water solubility, low vapor pressure, and similar melting and boiling points; pp. 4, 21); and (iii) transport, biodegradation and toxicity (as far as is known, the compounds are similar in these respects). In preparing this categorization, HERTG has followed the six steps advocated by the EPA (test plan, p. 3).

(c) *It takes the low exposure risk into consideration*

DAEs are closed-system intermediates, which are not transported, are manufactured in closed reaction tanks, and are transferred in closed-system pipes. Therefore, although acute human exposure (most probably dermal) can occur in the event of spillage, chronic or subchronic exposure is highly unlikely. Repeat-dose, reproductive and developmental studies are therefore not necessary (test plan, p. 17), and are in fact prohibited by the October 1999 Agreement among animal protection organizations, EPA, and CMA (Item 7: "Participants shall not develop sub-chronic or reproductive toxicity data for the HPV chemicals that are solely closed system intermediates").

For the above reasons, HERTG has appropriately decided that no further mammalian tests are needed. However, HERTG has included in the test plan a fish toxicity test (OECD guideline 203), to be carried out with CAS no. 84605-28-7 (test plan, p. 11). This test will kill at least 60 fish. Because DAEs have low aqueous solubility, the intention is to carry out the test using the static-renewal method, which involves replacing the solution every 24 hours (test plan, p. 10).

<u>There are three overwhelming reasons why the fish test is inappropriate in this case</u>:

1. Fish data are already available

The test plan contains the following statement: "There are no published or unpublished fish acute toxicity data for members of the dithiophosphate alkyl esters category" (p. 11). However, this statement is incorrect, as CAS no. 68649-43-4 has been tested on the fathead minnow (*Pimephales promelas*; O'Boyle, 1987). The 96-hour LC_{50} concentration was 9.2 mg/L, and the 96-hour acute no-observed-effect concentration was 5.0 mg/L.

2. DAEs are too hydrophobic for fish tests

The EPA has stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2, and recommends that with such highly hydrophobic compounds a chronic *Daphnia* test be used instead of acute fish and *Daphnia* tests (EPA, *Federal Register* 2000, p. 81695). The log $K_{o/w}$ values of the DAEs are predicted to be in the range 4.48-7.99 (test plan, p. 4). Therefore, the fish toxicity test should not be conducted.

3. *In vitro* and *in silico* test methods are available

If HERTG wishes to investigate acute fish toxicity, we urge it to use one or more of the several available *in vitro* and *in silico* methods.

With respect to *in silico* methods, several quantitative structure activity relationship (QSAR) programs for estimating toxicity to fish and other aquatic organisms are available. The EPA itself encourages the use of one established QSAR: ECOSAR (EPA 2002).

With respect to *in vitro* methods, TETRATOX, an assay based on the protozoan *Tetrahymena pyriformis* (Larsen 1997), is the most appropriate. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997). The extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After more than one year, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for action rather than words.

The recently validated *Dar*T test is another prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, the *Dar*T test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

For the above reasons, there is no justification for carrying out a fish test, and we urge that it be removed from the test plan. A number of additional considerations relating to the inappropriateness of this fish test are detailed in the Appendix.

Thank you for your attention to these comments. I can be reached at 757-622-7382, extension 1304, or via e-mail at JessicaS@PETA.org.

Sincerely,

Jessica Sandler, MHS
Federal Agency Liaison
People for the Ethical Treatment of Animals

Richard Thornhill, PhD
Research Associate
PETA Research and Education Foundation

APPENDIX – ADDITIONAL CONSIDERATIONS

1. The ecologic relevance of fish toxicity should be taken into consideration

The difference between the purposes of the ecotoxicity and mammalian toxicity tests must be noted. The principle of the mammalian toxicity tests is that they are assumed to be useful for predicting toxicity in individual humans. Fish tests, on the other hand, are not for predicting toxicity in individual fish, but for predicting economic loss (to commercial and "sport" fisheries) and ecologic damage (fish are an important part of the food chain). The test therefore aims to show whether exposure with DAEs will result in large-scale fish death. However, water exposure can wipe out fish stocks even with no direct toxicity, because killing the food of the fish will lead to starvation. Carps and catfishes are herbivorous, eating mostly algae, whereas most other familiar North American freshwater fish species are carnivorous, eating worms, small crustaceans, smaller fish, insect larvae, etc. However, the toxicity of DAEs towards these types of organism is unknown, as shown by the inclusion in the test plan of tests on an aquatic crustacean (*Daphnia magna*) and a unicellular green alga (*Pseudokirchneriella subcapitata*). Fish tests should not be carried out while other types of aquatic toxicity are uncertain.

Physical fouling (below) is important in the context of the food chain, as it has particularly severe effects on phytoplankton, which directly or indirectly support most fish species (Hewstone 1994), and these effects could affect the need for an *in vivo* fish test.

2. DAEs may cause physical fouling

Water pollution with viscous, hydrophobic liquids such as DAEs often results in the physical fouling of aquatic organisms, such as gill coating in the case of fish. The liquids also often form a surface sheen, which can lead to oxygen deficiency (the specific gravity of the DAEs is not

stated, so it is unclear whether this will occur). It is therefore possible that DAEs will have greater fouling than toxic effect, in which case DAE toxicity is purely academic.

3. DAEs are associated with compounds with higher toxicity and high physical fouling potential

DAEs are closed-system intermediates. They are not transported or drummed, and remain within the vessel in which they are produced until they are converted to metal compounds (test plan, p. 5). Therefore, the only way they can enter the environment is from a spill within the manufacturing site. However, DAEs are only minor to moderate components of potentially spilled mixtures, and some of the other components probably have considerably higher toxicity. Therefore, the other components should drive the risk assessments of the mixtures, and DAE toxicity is of little importance.

The first type of spill is from the reaction tank alone. The reaction mixture usually includes phosphorus pentasulfide (test plan, pp. 3, 5, ACC 2002a, p. 4), which shows high acute toxicity in humans and rats (Payne 1993a, 1993b). The mixture also usually includes high-fouling-potential compounds, such as CAS numbers 64742-54-7 and 64741-88-4 (ACC 2002a, p. 4). These are viscous, hydrophobic and less dense than water (CONCAWE 1997), and the latter in particular is a component of products frequently responsible for fouling aquatic organisms (Shell 1999).

The second type of spill is a site-wide event, due to an explosion, a fire, an earthquake, a bombing, etc. It is this type of spill that would be most likely to result in environmental exposure to DAEs, because DAE reaction tanks are surrounded by dikes, and any spilled material is treated or removed for disposal (test plan, p. iii), so small spills should almost always be caught immediately. Site-wide spillage would necessarily result in simultaneous exposure to compounds with higher toxicity and fouling potential.

4. It is doubtful whether spillage would result in exposure of fish-inhabited water

There are four routes by which DAEs may enter fish-inhabited water from a spill within the manufacturing site:

(i) *Via groundwater*. This can be eliminated. Firstly, DAEs are hydrophobic, with very high octanol/water partition coefficients (test plan, p. 21), so they will partition predominantly into the particulate matter in the soil (test plan, p. 8). Secondly, they are highly viscous, so will not migrate readily through soil pores.

(ii) *Via the sewage system*. The importance of this route is reduced by the DAE tendency to partition to particles in sewage-treatment works. However, it also depends on biodegradability. The biodegradability of DAEs is not known, and no test for this parameter is included in the test plan, because it is expected to be approximately the same as the biodegradability of two zinc dialkyldithiophosphates, due to the molecules having the same hydrocarbon moiety (test plan, p. 6). Zinc dialkyldithiophosphates show only 4.2-5.9% biodegradability over 28 days (ACC 2002a, p. 8). However, the test

methods used involve measurement of biodegradation in solution. Therefore, although all or part of the inoculum added to the solution is supernatant from a sewage-treatment works (ACC 2002b, pp. 2-4), the zinc dialkyldithiophosphates do not have the opportunity to partition to a particulate phase, and the conditions are markedly unlike those obtaining in a real sewage-treatment works.

(iii) *By being washed by runoff, hosedowns, storm drains, etc*. Movement of DAEs over significant distances is limited by their viscosity. However, estimation of the probability of spilled DAEs entering fish-inhabited water depends on information that we do not possess about each manufacturing site, such as its layout, surrounding terrain, and climate.

(iv) *Directly into fish-inhabited water*. Reaction tanks are unlikely to be built immediately adjacent to fish-inhabited water, and direct spillage is therefore unlikely except due to a site-wide event. As discussed above, a site-wide event would release such a mixture of compounds, many far more toxic than DAEs, that DAE toxicity would be irrelevant.

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